The impact of intradialytic cycling on the removal of protein-bound uraemic toxins:

a randomised cross-over study

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ABSTRACT

The evidence on impact of intradialytic exercise on the removal of urea, is conflictive. Impact of exercise on kinetics of serum levels of protein-bound uraemic toxins, known to exert toxicity and to have kinetics dissimilar of those of urea, has so far not been explored. Furthermore, if any effect, the most optimal intensity, time point and/or required duration of intradialytic exercise to maximise removal remain obscure. We therefore studied the impact of different intradialytic cycling schedules on the removal of protein-bound uraemic toxins during haemodialysis (HD).

This randomised cross-over study included 7 stable patients who were dialysed with an FX800 dialyser during three consecutive midweek HD sessions of 240min: A) without cycling; B) cycling for 60 minutes between 60^{th}-120^{th}min of dialysis; and C) cycling for 60 minutes between 150^{th}-210^{th}min, with the same cycling load as in session B. Blood and dialysate flows were respectively 300 and 500mL/min. Blood was sampled from the blood inlet at different time points, and dialysate was partially collected (300mL/h). Small water soluble solutes and protein-bound toxins were quantified and intradialytic reduction ratios (RR) and overall removal were calculated per solute.

Total solute removal and reduction ratios were not different between the 3 test sessions, except for the reduction ratios $RR_{60\text{-}120}$ and $RR_{150\text{-}210}$ for potassium.

In conclusion, we add evidence to the existing literature that, regardless of the timing within the dialysis session, intradialytic exercise has no impact on small solute clearance, and demonstrated also a lack of impact for protein-bound solutes.

INTRODUCTION

During the past decennia it became more obvious that, apart from successive improvements in commercially available haemodialysers, one should focus more on the patient to increase dialysis adequacy [1, 2]. Dialysis strategies such as more frequent and more prolonged dialysis have proven benefit for the removal of numerous uraemic toxins [3, 4]. While different changes in dialysis strategy might have some beneficial effects on removal of small water soluble solutes and middle molecules [3, 5-7], only processing larger volumes of blood on a weekly basis seems to have a beneficial impact on the removal of protein-bound toxins [8]. Since this can only be obtained by substantially increasing dialysis time per week, this option is less or even not suitable for regular in-centre haemodialysis. As not all patients are candidates for nocturnal or home dialysis, other alternative tracks to enhance protein-bound toxins are being investigated [9, 10].

Already in the late 90's, intradialytic exercise has been promoted to increase small solute based dialysis adequacy [11]. While different studies showed improved reduction ratios and/or solute removal for urea [12-17], several more recently performed studies could however not confirm these findings [18-20]. A systematic review of the current literature thus described a lack of efficacy for intradialytic exercise to enhance urea clearance [21]. However, urea is not representative for the kinetics of other small and water soluble toxins, such as phosphate [22-24]. For this molecule, intradialytic exercise appeared to be efficacious at improving its removal [12, 19, 20, 25]. Especially for protein-bound uraemic toxins, kinetics are substantially dissimilar from those of urea [8, 26]. The improved muscle perfusion during cycling might increase the release of free fraction of protein-bound uraemic toxins from the deeper compartments in the body, changing the equilibrium between bound and free fractions, with eventually more removal of the free fraction. Furthermore, physical exercise may also augment intestinal microbial diversity through several mechanisms including the promotion of an anti-inflammatory state [27], lowering protein-bound toxin concentrations, and hence impacting removal. However, up to date, there are no data on the effect

of intradialytic exercise on the removal of these hard to remove toxins. There is also no consensus about the best time point, duration and intensity of cycling [21].

As a result, and despite the promising findings for phosphate, which could alleviate the pill burden, cycling during dialysis is not applied on a systematic basis in most dialysis centres, even not in those where bikes are available. A positive impact of intradialytic cycling on removal of protein-bound toxins, known to exert toxicity and to be related to outcome, could be a convincing argument to promote cycling during dialysis [28].

Therefore, the aim of the present study was to investigate 1) the impact of intradialytic cycling on the removal of protein-bound uraemic toxins as compared to small water soluble solutes, 2) how cycling influences the internal kinetics of uraemic toxins, and 3) whether timing of cycling within the dialysis session makes a difference.

METHODS

Patients

This is a prospective randomised cross-over study, designed according the Declaration of Helsinki, approved by the local Ethics Committee. Written informed consent was obtained from all participants (EC2014/0106 - B670201419836). Exclusion criteria were age below 18, a poor functioning vascular access, active infection, unstable cardiac condition, and not being able to bike for 60min. Fourteen patients were considered eligible for inclusion, and eight of these patients finally were enrolled in the study (figure 1). One of them could for medical reasons not attend one of the test sessions and was further excluded from all analyses. The remaining seven stable chronic haemodialysis patients (4 male, 1 with diabetes mellitus) were 68[45;75] years, spent 50±32 months on dialysis, had a body weight of 74±22 kg and a residual kidney function of 1.2[0.0;6.1] mL/min at the time of inclusion (table 1). Five patients had a well-functioning arteriovenous fistula as vascular access, i.e. fistula flow was higher than 600mL/min as measured with Blood Temperature Monitoring (BTM) during monthly follow-up, and two patients a Palindrome 14.5F central venous catheter (Covidien, USA).

Study design

During three consecutive weeks, the midweek session was studied. Haemodialysis was performed for at least 4 hours with an FX800 haemodialyser on a Fresenius 5008 dialysis machine (both Fresenius Medical Care, Bad Homburg, Germany). Blood and dialysate flows were set at 300 and 500mL/min, respectively, while ultrafiltration was set manually according to the patient's need. Ultrafiltration was kept constant during each test session, but could be different among the three sessions. Dialysate temperature was maintained at 36°C during the three midweek test sessions (A, B, and C). Session A was a dialysis session without any cycling (control), while during session B and C, the patient was asked to cycle for 60 minutes, but at different time points within the dialysis session: either from minute 60 to 120 or from minute 150 to 210 after dialysis start (figure 2), without a warm-up period. Biking was performed on a Motomed Letto 2 bike (Reck, Betzenweiler,

Germany), operated and installed by skilled dialysis staff. Patients were neither tested nor asked for their physical capacities and could choose for themselves the resistance and number of rotations but had to keep these fixed during both cycling intervals in sessions B and C.

Sampling and analysis

During the three midweek test sessions, blood was sampled from the vascular access predialysis, and from the blood inlet line at 15, 30, 60, 90, 120, 150, 180, 210, and 240min after the start of the session (figure 2). Blood samples were centrifuged (10min at 1250g, 4°C) after 20-30min at room temperature to allow clotting, after which the serum was aliquoted and stored at -80°C until batch analysis. Spent dialysate was partially collected during the entire session (300mL/h) using a calibrated sampling system (including an infuse pump type Alaris® GP plus; Cardinal Health, Switzerland).

Urea, creatinine (Crea), potassium (K), and phosphate (P) were measured by standard laboratory methods. Other solutes were determined by reversed-phase high performance liquid chromatography (RP-HPLC), as described earlier [29]. These included uric acid (UA, 168Da), and the protein-bound solutes p-cresyl glucuronide (PCG), hippuric acid (HA), indole acetic acid (IAA), indoxyl sulfate (IxS), and p-cresyl sulfate (PCS). To determine the total concentration, serum samples were first deproteinised by heat denaturation prior to HPLC analysis [29]. HA was analysed by UV detection at 254nm, and UA at 300nm, whereas *p*CG and *p*CS (λexc = 265nm, λem = 290nm) and IAA and IxS (λexc = 280nm, λem = 340nm) were determined by fluorescence detection [7, 30].

Measurements and calculations

During the three test sessions, accurate monitoring of pulse, blood pressure, blood volume (BVM: blood volume monitoring) and temperature (BTM: blood temperature monitoring) was performed using features on the dialysis machine, at the time points of blood sampling as well as every 15 minutes during cycling.

Percentage protein binding was calculated from the total C_T and free concentration C_F of the protein-bound uremic toxin as:

$$\%PB = \frac{C_T - C_F}{C_T} \cdot 100 \quad (1)$$

The bound concentrations of protein-bound toxins at time point t after dialysis start t_0 were corrected for haemoconcentration using BVM measurements at t and t_0 according to:

$$C_{t\_corr} = \left(C_t \cdot \frac{BVM_{t_0}}{BVM_t} \cdot \frac{\%PB}{100} \right) + \left(C_t \cdot \left(1 - \frac{\%PB}{100} \right) \right) \quad (2)$$

Reduction Ratios (RR - %) between two time points t_1 and t_2 (i.e. $RR_{0\text{-}240}$, $R_{60\text{-}120}$, and $R_{150\text{-}210}$) were calculated as:

$$RR = \frac{C_{t1} - C_{t2}}{C_{t1}} \cdot 100 \quad (3)$$

Total Solute Removal (TSR) was calculated from the processed dialysate volume (V_D) and the toxin concentration in the collected spent dialysate (C_D):

$$TSR = C_D \cdot V_D \quad (4)$$

Statistical analysis

All statistical analyses were performed using SPSS Statistics 25 (SPSS Inc, Chicago, IL). Data are expressed as mean ± standard deviation or median[25^{th};75^{th} percentile]. Statistical analyses were carried out using the repeated measures ANOVA with post hoc Bonferroni.

RESULTS

For the seven patients completing the test protocol as foreseen, all data of the three randomised mid-week dialysis sessions were used in the analysis (figure 1). Also, no side effects were revealed during the test sessions. For the only patient dialysing longer than 240min (i.e. 270min), the last test sample and data were collected at 240min. Ultrafiltration volumes were not different among the three different test sessions, respectively 1.36±0.94L, 1.43±1.09L, and 1.14±1.11L. Patient's medication and bicarbonate dialysate concentrate were not changed during the three test weeks (table 1).

Table 2 shows the cycling characteristics of the seven patients which were similar for dialysis sessions B (cycling from 60-120min) and session C (cycling from 150-210min). All patients performed the cycling according to their physical possibilities, explaining the wide variety for the achieved resistance as well as the frequency of rotations (i.e. suppleness).

The mean serum concentrations during the three dialysis sessions are presented in figure 3 for the protein-bound solutes and in figure 4 for the small water soluble solutes, with the data for sessions A, B, and C in the respective panels. While most toxins show an exponential-like concentration decrease during the course of dialysis, potassium and phosphate concentrations tended to increase during cycling in session B (potassium only) and C (figure 4).

Table 3 shows the reduction ratios for the three test sessions, as calculated over the total 4h dialysis session ($RR_{0\text{-}240}$), and over both 60min cycling intervals (i.e. $RR_{60\text{-}120}$ and $RR_{150\text{-}210}$). Cycling did neither affect the reduction ratios of the protein-bound toxins nor of the small water soluble solutes, apart from potassium. For the latter, $RR_{60\text{-}120}$ (ANOVA P=0.025) was significantly lower and even became negative during the cycling interval (session B) as compared to sessions A (P=0.012) and C (P=0.043). Also $RR_{150\text{-}210}$ for potassium (ANOVA P=0.005) was lower and even negative during the cycling interval (session C) compared to the corresponding reduction ratio in session A (P=0.007) and B (P=0.002).

For none of the studied solutes, differences in Total Solute Removal (TSR) could be measured (figure 5).

Although several individual fluctuations could be observed in the relative blood volume (BVM) during the dialysis course, blood volume variation from start to end of dialysis was not different among the three dialysis sessions.

Several registered parameters, i.e. pulse, blood pressure, and temperature, illustrated that the intensity of cycling in sessions B and C were comparable, and that baseline parameters were similar for all sessions.

At rest, pulse was not different between the different sessions: i.e. 67±3bpm (session A) versus 67±4bpm (B) and 66±2bpm (C). Pulse increased significantly during cycling in session B (median pulse 82±10bpm and top 102±26bpm) and session C (median 84±7bpm and top 94±16bpm), but no differences were found for the median and top pulses between session B and C.

In a similar way, systolic blood pressure at rest was not different between the three sessions (i.e. 134[85;141]mmHg in session A, 124[88;138]mmHg in session B, and 120[94;150]mmHg in session C), and no differences were observed for the mean and top systolic blood pressure during cycling between session B and C (i.e. mean 128[93;150] and top 135[109;156]mmHg in session B, and mean 143[93;155] and top 164[102;173]mmHg in session C).

Blood temperature, as registered by BTM, increased during the cycling interval by 0.5±0.2°C (session B) and 0.5±0.4°C (session C). Post dialysis, blood temperature was still 0.3±0.4°C (session B) and 0.4±0.2°C (session C) higher than at 15min after dialysis start, and these increases were significantly higher than the 0.2±0.3°C increase after the session without cycling.

DISCUSSION

The recently performed systematic review by Kirkman et al. was clear in its conclusion [21]: while it remains uncertain whether intradialytic exercise can improve dialysis adequacy in terms of urea removal, the impact on the removal of the more toxic protein-bound toxins as well as the evidence of effective exercise prescription parameters, are still unexplored. The present study focused on the effect of cycling during dialysis on removal of protein-bound toxins, and assessed whether timing of cycling during the dialysis session had an impact. Our main findings are that, (1) intradialytic cycling did not improve the removal of protein-bound toxins, and this irrespective of timing within the dialysis session; (2) no improvement in the removal of urea was observed; and (3) potassium serum concentrations were increased during the cycling interval but this did not result in enhanced removal.

The underlying hypothesis of intradialytic exercise increasing dialysis adequacy, includes increased blood perfusion between the working muscle and the bloodstream, thereby increasing the transport of toxins to the plasma and thus enabling more toxin removal during dialysis. In case this enhanced intercompartmental transport is higher than dialyser removal, plasma concentrations might even increase during cycling. From this respect, the reduction ratio, as calculated from pre and post dialysis plasma concentrations, is no overall suitable marker to quantify the impact of cycling. On the contrary, total solute removal, as calculated from spent dialysate volume and concentrations, provides a more objective way to quantify the impact of cycling. The present finding that protein-bound uraemic toxins do not take advantage of this enhanced transport inside the patient, teaches us that either the release of these toxins into the plasma is very limited or that the (immediate) protein binding of the solute released into the plasma prevents its removal. Also, the variation of gut microbiota, influencing PBUT concentrations and removal, might only become important on the long run when exercise is performed on a regular basis.

Many contradictory results on the impact of intradialytic cycling on urea removal have been published over the last two decades [11-18, 20, 25, 31]. In our study, no impact of cycling was found. Most

studies showing an increased Kt/V_{urea} were RCTs running over several weeks (8-20 weeks) [14-17]. This suggests that the improvement of Kt/V_{urea} with cycling might be more the result of a long term effect, such as improved body composition by reduction of extracellular water or improved nutritional status[32]. Unfortunately, many of the cross-over studies finding a positive impact are rather old and lack sufficient details to develop or unravel potential mechanistic hypotheses [11-13]. And this is also the case for the more recently performed studies which share the same conclusions as the present study [18, 20].

In the present study, we observed only for potassium an impact of intradialytic exercise on solute kinetics. The negative reduction ratios during both cycling intervals can be explained by the release of potassium ions from the skeletal muscles leading to increased plasma concentrations [33]. These higher concentrations should theoretically result in higher potassium removal via the dialyser. However, according to our results, this did not happen. It can be that the vigorous exercise induced neuro-hormonal activation, resulting in a rapid shift of potassium to intracellular once exercise is stopped, and thus a lower removal in the post-exercise period which would compensate the higher removal during exercise [34]. This could be the case during the performance of session B, where patients have a substantial period of dialysis after the exercise, but of course far less during session C, where this period is shorter [34]. Although the observed temporary hyperkalaemia during cycling is depending on the exercise load [13, 35], our findings with respect to the lack of an increased potassium removal are still in accordance to those described by Orcy et al. where patients had to cycle three times 20min even at a rather strong level up to 80% of their maximum heart rate [20].

Rather surprisingly, also no increase in phosphate total solute removal was observed in the present study. From the complex kinetics of phosphate [22-24] it is clear that its removal is taking advantage of prolonged dialysis, as displacement from third compartments to the circulation is the rate limiting step for removal [36]. From the same reasoning it could be hypothesised that the mobilisation of phosphate from the deeper compartments is enhanced during intradialytic exercise. This advantage only applies when the exercise is performed either in a moderate way every dialysis hour for at least

20min [12, 25] or rather intensive during the last hour of the dialysis session [19]. On the contrary, exercise as scheduled during the first hours of dialysis seemed not to increase phosphate removal [20, 31]. The present study adds to these findings that moderate active cycling in the interval 60-120 or 150-210min after dialysis start did not result in enhanced phosphate removal.

The small patient number (n=7) could be considered a limitation of the study, but this allowed us to have each patient as his/her own control over the 3 experimental regimens. While several other studies are defining the exercise intensity as a percentage of maximum heart rate or anaerobe threshold, we allowed the patients to choose their own exercise load to make the results more relevant for daily clinical practice. Also, although the physical exercise of some patients could be defined as rather limited, a sub-analysis including the four patients using a cycling resistance above 5 (table 2) did not change the results. However, in order to prescribe individualised physical activity with a formal treatment plan, it is not sufficient to follow the patient's choice. Instead, exercise professionals should assess the patient's capabilities beforehand and should document their progress [37, 38].

But our study has also several strengths. We are the first to check the kinetics of protein-bound uraemic toxins during intradialytic exercise, and this with a rather long exercise interval (60min) in the first (i.e. 60-120min) as well as in the second part of the dialysis session (i.e. 150-210min). Furthermore, the results of both sessions with cycling could be compared in a direct way, due to the prescription of an equal exercise load, as is also reflected by the outcome of other registered parameters during cycling like pulse, blood pressure and temperature. The intensive blood sampling during dialysis, with at least samples pre and post cycling, offered the opportunity to check intradialytic kinetics, i.e. $RR_{60\text{-}120}$ and $RR_{150\text{-}210}$. While reduction ratios do not reflect the net gain in toxin removal by intradialytic cycling, the measurement and calculation of total solute removal is a strong added value of this study.

In conclusion, although our study was designed to learn more about the intradialytic kinetics of a whole range of uraemic toxins, we could not find any difference in removal between the sessions

with and without cycling, nor for the protein-bound uraemic toxins, neither for the small water soluble toxins. Hence, internal kinetics of uraemic toxins was not found influenced by intradialytic cycling, and no recommendations could be defined with respect to the best timing interval for cycling.

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TABLES

Table 1: Patient and dialysis characteristics

Included patients (n)	7
Patient characteristics	
Gender (M/F)	4/3
Age (years)	68 [45;75]
Body weight (kg)	74 ± 22
BMI (kg/m²)	26 ± 7
RKF (mL/min)	1.2 [0.0;6.1]
Dialysis vintage (months)	50 ± 32
Etiology	myeloma kidney, tuberous sclerosis, membranous nephropathy, diabetic nephropathy in diabetes type II, atheroembolic renal disease, (acute) cortical necrosis, glomerulonephritis
Medication	calcium acetate (n=4), sevelamer (n=1), calcium kayexalate (n=1), sodium bicarbonate (n=1), alfacalcidol (n=2), cholecalciferol (n=1), pantoprazole (n=2), acetylsalicylic acid (n=2), simvastatin (n=1), dopidogrel (n=1), bumetanide (n=1), amplodipine (n=1), carvedilol (n=1), cyclofosfamide (n=1), fludrocortisone (n=1), finasteride (n=1), trazodan (n=1)
Dialysis characteristics	
Anticoagulation	Heparin 4000IE + 1500IE/h (n=1), Tinzaparin 4500IE (n=3), Tinzaparin 3500IE (n=1), Enoxaparin 60mg (n=2)
Bicarbonate dialysate concentrate	K4 Ca1.5 (n=1), K3 Ca1.5 (n=1), K3 Ca1.25 (n=3), K2 Ca1.25 (n=1), K2 Ca1.25 (n=1)
Laboratory	
Hb (g/dL)	10.7 ± 1.5
CRP (mg/L)	5.3 [3.0;27.2]
Na (mmol/L)	141 ± 1
K (mmol/L)	5.0 ± 0.6
Ca (mmol/L)	2.1 ± 0.2
P (mmol/L)	1.4 ± 0.4
Urea (mg/dL)	84.6 ± 12.6
Creatinine (mg/dL)	7.5 ± 1.6
Albumin (g/L)	38.8 ± 4.6
Total protein (g/L)	62.3 ± 4.0
PTH (ng/L)	231 [166;361]
Vit D (ng/mL)	15.2 [12.2;24.4]

Table 2: Cycling characteristics per patient.

Patient	Resistance	Rotations/min
1	0	75
2	6	90
3	6	80
4	10	60
5	4	80
6	15	90
7	4	90

Table 3: Reduction ratios for different time intervals during dialysis for the different sessions A, B, and C.

Solute	**RR_{0-240}**			**RR_{60-120}**			**$RR_{150-210}$**		
	A	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
Protein-bound uraemic toxins									
PCG	78 ± 11	78 ± 13	72 ± 32	35 ± 8	33 ± 6	31 ± 15	25 ± 13	23 ± 15	20 ± 24
HA	69 ± 8	70 ± 12	67 ± 19	27 ± 8	26 ± 8	27 ± 5	22 ± 8	22 ± 2	22 ± 8
IAA	44 ± 8	43 ± 9	51 ± 10	8 ± 10	11 ± 8	14 ± 5	12 ± 7	9 ± 6	13 ± 7
IxS	34 ± 5	32 ± 9	38 ± 8	8 ± 8	6 ± 11	8 ± 4	7 ± 6	6 ± 6	8 ± 5
PCS	29 ± 4	28 ± 7	32 ± 8	6 ± 6	6 ± 11	7 ± 4	6 ± 5	5 ± 5	5 ± 4
Small water soluble solutes									
Urea	78 ± 3	78 ± 4	77 ± 4	30 ± 3	27 ± 2	29 ± 3	29 ± 4	29 ± 4	27 ± 3
Crea	70 ± 3	70 ± 5	69 ± 4	23 ± 4	21 ± 3	23 ± 2	21 ± 2	21 ± 4	18 ±3
P	59 ± 5	61 ± 7	63 ± 6	14 ± 14	13 ± 8	22 ± 3	1 ± 14	-1 ± 11	-11 ± 9
K	22 ± 12	20 ± 10	24 ± 14	***5 ± 3*** $^{\alpha}$	***-7 ± 6****	***5 ± 6***†	***1 ± 3*** $^{\alpha}$	***2 ± 4***	***-9 ± 3**†***
UA	79 ± 6	79 ± 5	78 ± 4	31 ± 4	30 ± 4	31 ± 3	26 ± 5	27 ± 4	26 ± 5

RR: reduction ratio; PCG: p-cresyl glucuronide; HA: hippuric acid; IAA: indole acetic acid; IxS: indoxyl sulfate; PCS: p-cresyl sulfate; P: phosphate; K: potassium; UA: uric acid.

$^{\alpha}$ repeated measures ANOVA P<0.05

* P<0.05 versus A; † P<0.05 versus B

FIGURES

Figure 1: Flow chart of patient inclusion

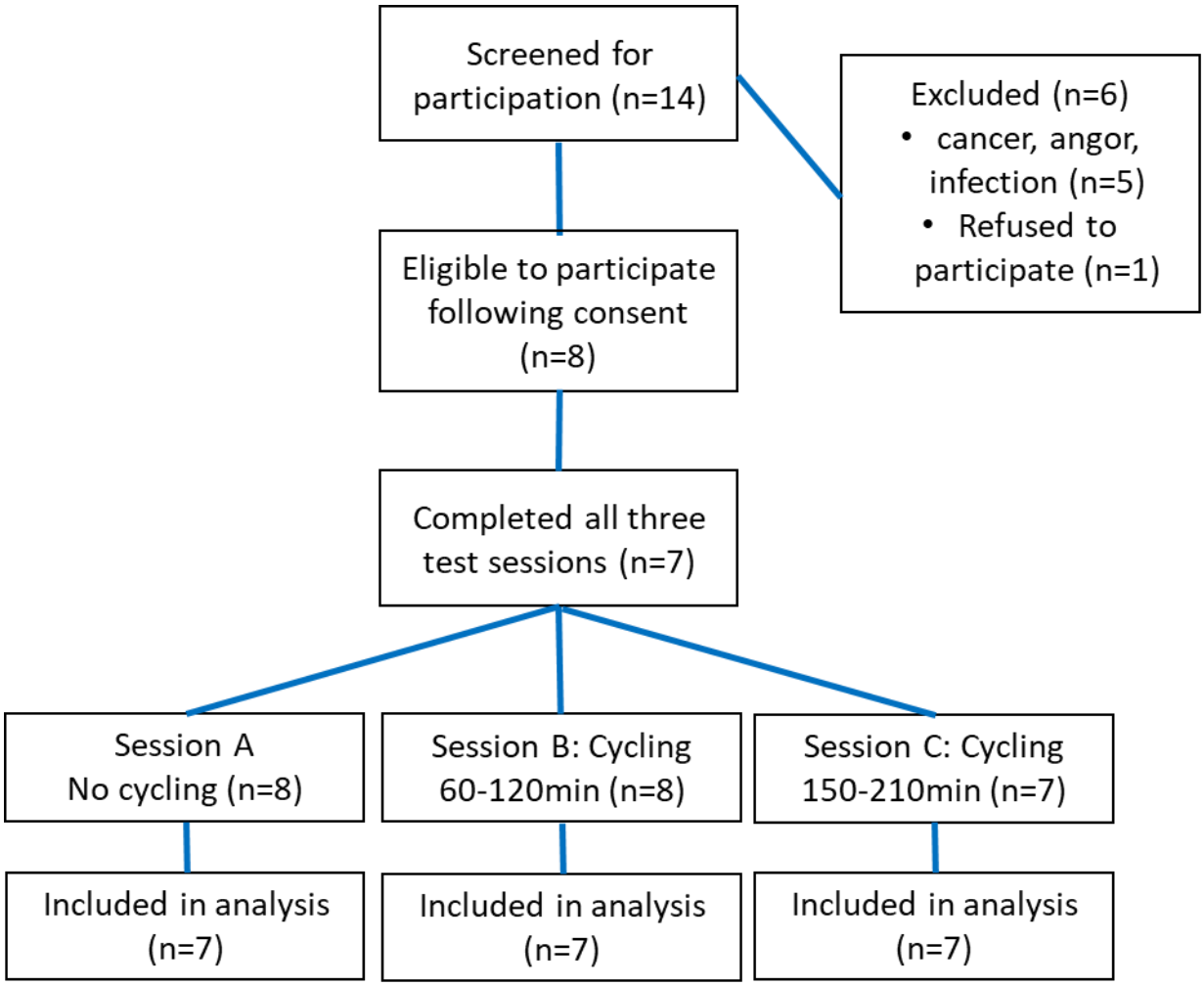

Figure 2: Protocol of cycling (grey bars) and blood sampling (arrows) in the three midweek sessions A, B, and C.

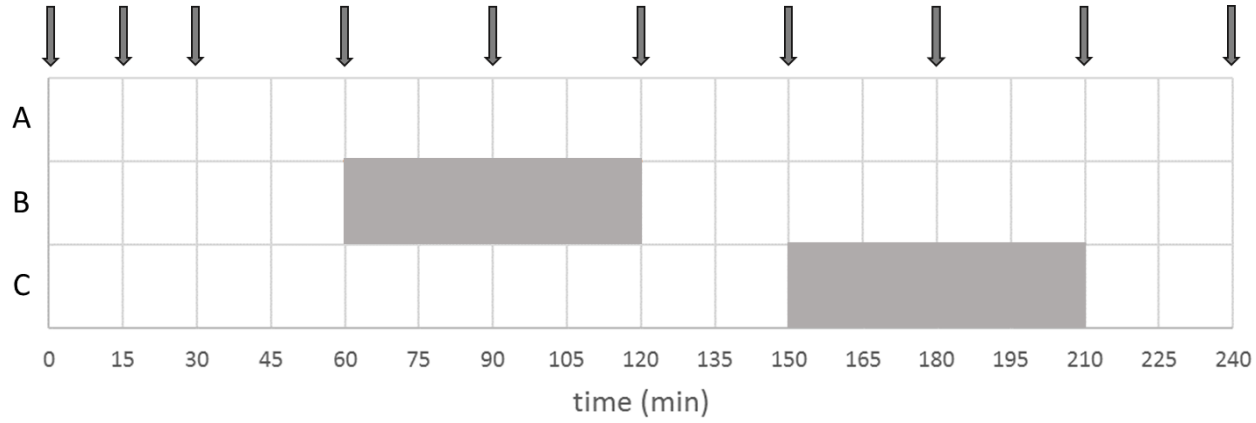

Figure 3: Profiles of mean concentrations of protein-bound uraemic toxins along the session without cycling (session A - panel A), with cycling from 60-120min (session B - panel B), and with cycling from 150-210min (session C - panel C). Cycling intervals are indicated in grey. IxS: indoxyl sulfate, PCS: p-cresyl sulfate, PCG: p-cresyl glucuronide, IAA: indole acetic acid, and HA: hippuric acid. N=7

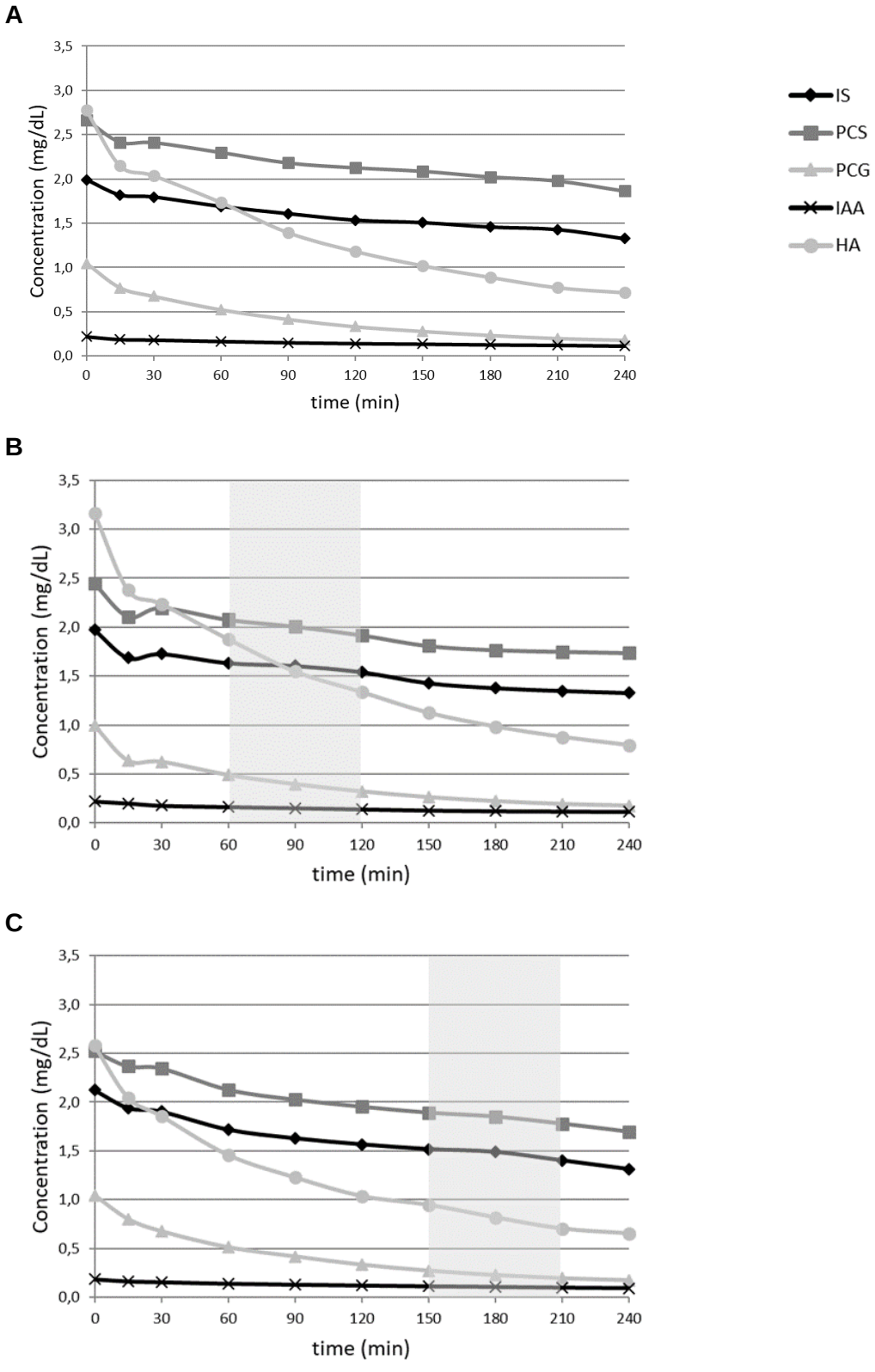

Figure 4: Profiles of mean concentrations of small water soluble solutes along the session without cycling (session A - panel A), with cycling from 60-120min (session B - panel B), and with cycling from 150-210min (session C - panel C). Cycling intervals are indicated in grey. Crea: creatinine, P: phosphate, K: potassium, UA: uric acid. N=7

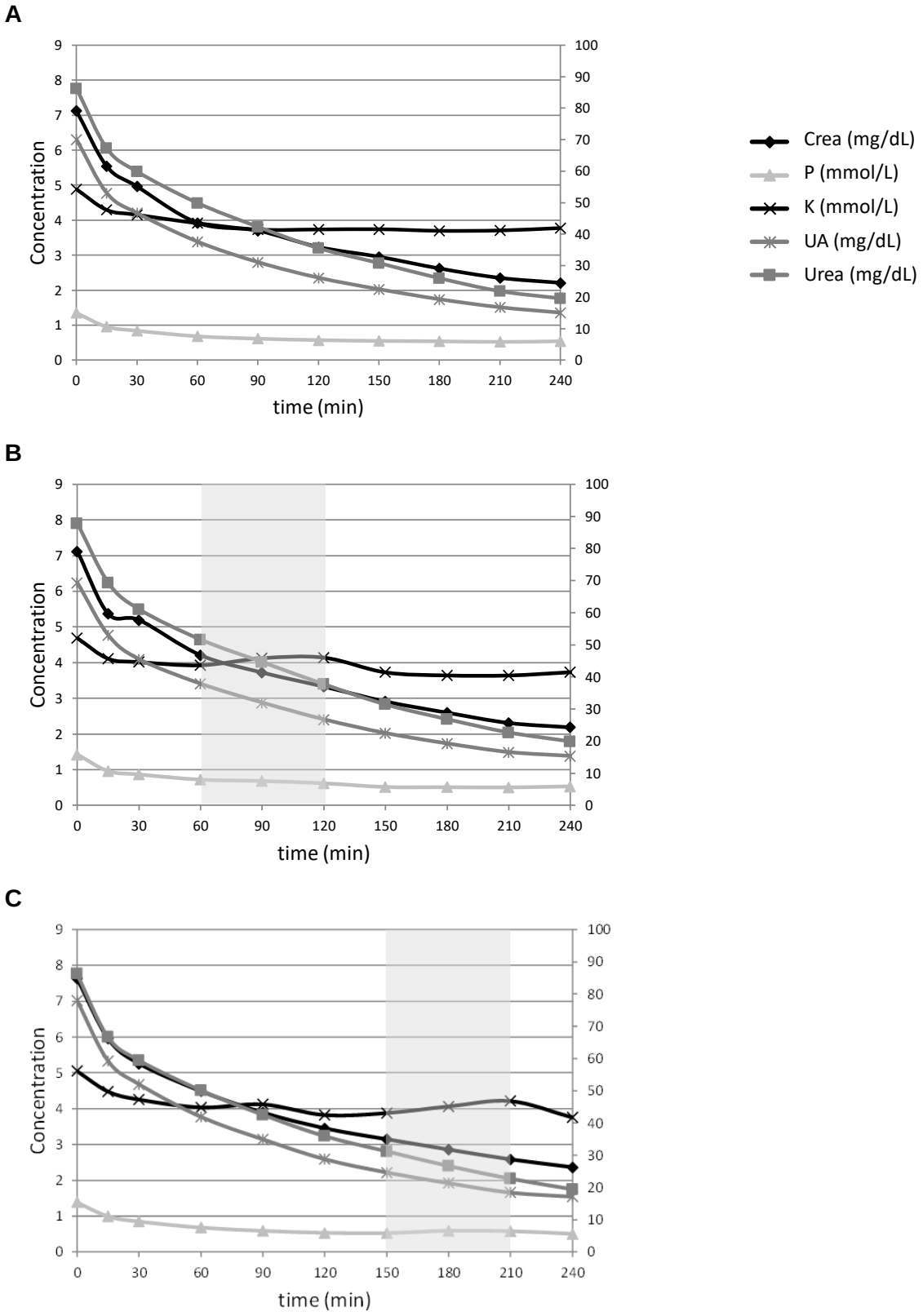

Figure 5: Total Solute Removal (TSR) of small water soluble solutes (panel A) and protein-bound solutes (panel B) in the three midweek sessions A, B, and C. Crea: creatinine, P: phosphate, K: potassium, UA: uric acid, PCG: p-cresyl glucuronide, HA: hippuric acid, IAA: indole acetic acid, IxS: indoxyl sulfate, PCS: p-cresyl sulfate. N=7